September 29, 2005

Participant
Centers for Disease Control and Prevention (CDC)
*Mycobacterium tuberculosis* Nucleic Acid Amplification Testing
Performance Evaluation Program

Subject: Analyses of Participant Laboratory Results for the June 2005 Shipment

Dear Participant:

Enclosed are analyses of laboratory test results reported to the Centers for Disease Control and Prevention (CDC) by participant laboratories for the June 2005 shipment of samples for the CDC *Mycobacterium tuberculosis* Nucleic Acid Amplification (*M.tb* NAA) Testing Performance Evaluation Program. Participant laboratories received five individual samples. Responses were received from 90 of 91 (99.0%) enrolled laboratories that received this shipment.

The enclosed aggregate report is prepared in a format that will allow laboratories to compare their results with those obtained by other participants for the same sample using the same *M.tb* NAA test method.

We encourage you to circulate this report to all personnel involved with *M.tb* NAA testing, interpreting, or reporting. If you have any comments or suggestions on the format selected for the results, or questions regarding this report, you may call Laurina Williams at (770) 488-8130.

Sincerely yours,

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Enclosures

**Analyses of the June 6, 2005 Performance Evaluation Results for *M. tuberculosis* Nucleic Acid Amplification Testing Reported to the Centers for Disease Control and Prevention**

## Overall Summary of Results

***M.tb* positive and negative samples:**

| | | | 3 Positive Samples TB05-06-1 TB05-06-2 TB05-06-5 | 2 Negative Samples TB05-06-3 TB05-06-4 | |
|---|---|---|---|---|---|
| **Method** | **Total # of laboratories** | **Total # of results** | **False-negative results** | **False-positive results** | **Overall Performance** |
| Gen-Probe MTD | 70 | 350 | 3/210 (1.4%) | 0/140 (0.0%) | 99.1% |
| Roche Amplicor | 13 | 65 | 1/39 (2.6%) | 0/26 (0.0%) | 98.5% |
| In-house/Other | 7 | 35 | 4/21 (19.0%) | 1/14 (7.1%) | 85.7% |

## New Findings

- Participants did well in this shipment; overall accuracy was 98.0% (441/450).

- Six of ninety laboratories (6.7%), reported false negative interpretations for sample TB05-06-5. Three of seven participants using In-house methods (42.9%) reported false negative interpretations for this sample which contained a low concentration of *M.tb* ($3.0 \times 10^3$ theoretical cells/ml).

- A sample containing *M. mucogenicum* ($3.0 \times 10^3$ theoretical cells/ml), was included in this shipment. All participating laboratories, 100% (90/90) reported the correct interpretation as negative.

- One laboratory using an In-house method reported a false positive for Sample TB05-06-4, containing *Haemophilus influenzae* ($3.0 \times 10^4$ theoretical cells/ml).

- Of the participating laboratories, 6.9% (6/87) reported that they do not use uni-directional workflow. This is a decrease from the previous shipment indicating that some laboratories are using better practices.

## Note:

- It is a concern that 12.4% (11/89) of participants reported using Biosafety Level 2. (One laboratory reported that they do not know their Biosafety Level.) Please refer to CDC/NIH manual, Biosafety in Microbiological and BiomedicalLaboratories ($4^{th}$ edition), to determine the correct level of biosafety for your laboratory.

## Introduction

This report is an analysis of laboratory test results reported to the Centers for Disease Control and Prevention (CDC) by participant laboratories for the samples containing *M. tuberculosis* or non-tuberculous mycobacteria shipped in June 2005. Responses were received from 90 of 91 (99.0%) laboratories participating in this shipment. The *M.tb* NAA Performance Evaluation Program provides laboratories with a tool for external quality assessment. To maintain participant confidentiality, the CDC analyzes only participant data from which all laboratory identifiers have been removed by the contractor, Wisconsin State Laboratory of Hygiene (WSLH).

## Challenge Samples

Participant laboratories received five individual samples. The negative samples in this shipment were *H. influenzae* ($3.0 \times 10^4$ theoretical cells/ml) and *M. mucogenicum* ($3.0 \times 10^3$ theoretical cells/ml). Participants were requested to test the samples without the decontamination and concentration procedures routinely performed on respiratory specimens prior to *M.tb* NAA testing. The specimen decontamination/concentration preparation steps for *M.tb* NAA testing were eliminated to allow this program to specifically assess *M.tb* NAA testing procedures (2,6).

Experiments were performed to document sample viability and test reactivity. Due to specific concerns of cross-contamination between *M.tb* NAA-positive and *M.tb* NAA-negative test samples, the negative samples were produced in a separate area. Additionally, 10% of both positive and negative samples were randomly selected and tested by the contractor to validate *M.tb* NAA results. The samples were also tested by five reference laboratories before shipping.

## Results

Figure 1 shows the laboratory classification represented by 89 participants. Participants consisted of 38 hospitals, 40 health departments, 10 independents, and 1 other type of laboratory.

Figure 2 provides the distribution of the volume of specimens tested with *M.tb* NAA by participating laboratories during the 3 months prior to reporting results.

Figure 3 provides a breakdown of the *M.tb* NAA test procedures reported by the participating laboratories. Participants were asked to check all test methods used. All of the participants (7/7) reporting the use of In-house *M.tb* NAA test procedures used methods based on polymerase chain reaction (PCR). Although the CDC does not recommend the use of non-FDA cleared *M.tb* NAA test procedures (3,5), laboratories using In-house methods are encouraged to participate in this evaluation program to assess performance (2).

Figure 4 lists the biosafety levels reported by participant laboratories. All laboratories should routinely consult the CDC/NIH manual, Biosafety in Microbiological and Biomedical Laboratories ($4^{th}$ edition), for recommendations and for determining their correct biosafety level.

Figure 5 provides the participant laboratory responses to a question about whether the biological safety cabinet (BSC) used for *M.tb* NAA testing is used for other purposes.

It is a concern that 14.8% (13/88) of participant laboratories indicated that they process *M.tb* specimens in the same BSC that is used for *M.tb* NAA testing. This percentage has increased since the January 2005 shipment 12.2% (11/90). Among the 30.7% (27/88) of participants that indicated AOther@ uses for the *M.tb* NAA testing BSC, 13 performed *M.tb* testing procedures or culture work (biochemicals, drug susceptibility testing, Accuprobe7 identification, etc.), 10 performed mycology, and 4 performed other microbiology or clinical specimen work. One laboratory reported using the same BSC for bioterrorism-related work. Laboratories should be aware of recommendations (4) to perform specimen processing and NAA testing in separate work areas with separate equipment to avoid contamination problems.

Figure 6 provides participant responses to a question on the use of uni-directional workflow for *M.tb* NAA testing. In addition to recommendations (4) that emphasize considerations of laboratory design for NAA testing, both manufacturers (Roche Amplicor7 and Gen-Probe7 MTD) recommend the use of unidirectional workflow. It is a concern that 6.9% (6/87) of responding laboratories reported that unidirectional workflow is not being used or that they do not know if it is being used.

Separate figures and tables are provided to show either the qualitative or quantitative results reported for each sample by the participant laboratories. Quantitative results for the In-house methods could not be presented in a consistent format since participants used a variety of detection systems and test interpretation criteria. The Roche Amplicor7 test has interpretive criteria for quantitative results that reflect some probability that the sample is positive but is below the recommended threshold for positivity. The result form and this report use the term "equivocal" for Roche Amplicor7, to reflect the manufacturer=s recommendation for reporting indeterminate quantitative test results.

Figure 7 provides a summary of the participant qualitative results reported for all five samples by test method. The aggregate participant qualitative results are indicated for the 3 positive and 2 negative samples. The combined analytical sensitivity of all methods was 97.0% (262/270) for the TB05-06-1 ($3.0 \times 10^5$ theoretical cells/ml), TB05-06-2 ($3.0 \times 10^5$ theoretical cells/ml) and TB05-06-5 ($3.0 \times 10^3$ theoretical cells/ml): 98.6% (207/210) sensitivity for Gen-Probe7 MTD; 97.4% (38/39) sensitivity for Roche Amplicor7; 81.0% (17/21) sensitivity for In-house methods. The combined analytical specificity of all methods was 99.4% (179/180) for the 2 negative samples, *M. mucogenicum*, TB05-06-3, ($3.0 \times 10^3$ theoretical cells/ml) and *H. influenzae*, TB05-06-4, ($3.0 \times 10^4$ theoretical cells/ml): 100.0% (140/140) specificity for Gen-Probe7; 100.0% (26/26) specificity for Roche Amplicor7; 92.9% (13/14) specificity for In-house methods.

Figure 8 is graphical representation of the quantitative results reported for each sample by participant laboratories using the Gen-Probe7 MTD test. The indention in each box-plot indicates the median value. The shaded area within the box represents the results between the $25^{th}$ percentile and $75^{th}$ percentile of the data. The bracketed areas designate either 1.5 times the interquartile range of the data or the most extreme data point on either side of the median, whichever is the least distance from the median.

Each Gen-Probe7 value reported which was outside these ranges is signified by one of the solid lines drawn outside the brackets. For the positive samples, TB05-06-1, TB05-06-2 and TB05-06-5 the median values of all data were 3,285,504, 3,336,187 and 3,134,805 relative light

units (RLU), respectively. The median values for the negative samples containing *M. mucogenicum,* TB05-06-3, and *H. influenzae,* TB05-06-4, were 2,768 and 2,622 relative light units (RLU) respectively, similar to median values for other negative samples previously used in the program.

Figure 9 is a graphical representation of all quantitative results reported for each sample by participant laboratories using the Roche Amplicor7 test. The solid line through each set of data represents the median value for each sample. The shaded band represents the equivocal range. The median value for positive samples, TB05-06-1, TB05-06-2 and TB05-06-5 were 3.679 ($A_{450}$), 3.697 ($A_{450}$) and 2.395 ($A_{450}$) respectively. The median values for the negative samples containing *M. mucogenicum,* TB05-06-3, and *H. influenzae,* TB05-06-4, were 0.045 ($A_{450}$), and 0.044 ($A_{450}$) respectively. These median values are similar to results for other negative samples previously used in the program.

## Discussion

Most of the errors observed in this shipment were made by laboratories using In-house methods. These laboratories reported 19.0% (4/21) false negative and 7.1% (1/14) false positive errors. Three of seven laboratories (42.9%) reported false negative results using In-house methods for sample, TB05-06-5. This sample contained ($3.0 \times 10^3$ theoretical cells/ml) of *M. tuberculosis,* a concentration which was two orders of magnitude less than the other positive samples. Laboratories using In-house methods, should review their protocol or testing procedures to ensure accurate testing results.

Sample TB05-06-3, *M. mucogenicum,* was a new mycobacterium that was added to the Model Performance Evaluation Program. This organism was cultured from a patient at WSLH. All participating laboratories 100% (90/90) using all methods reported the correct interpretation as negative.

*M. mucogenicum,* formerly a Mycobacterium chelonei-like organism (MCLO), is a rapidly growing, nonpigmented waterborne mycobacterium (8). The frequent presence of this organism in tap water may result in transient colonization or contamination of sputum samples; therefore, a single positive sputum culture is usually not clinically significant. *M. mucogenicum* has been associated with peritonitis in patients on chronic peritoneal dialysis. Infections in two outbreaks were traced to contamination of the dialysis machines (9). *M. mucogenicum* has also been associated with skin and wound infections, and catheter-related sepsis (8).

Eleven out of eighty-nine laboratories (12.4%) reported using Biosafety Level 2 (One laboratory reported that they do not know their Biosafety Level.) This has raised questions regarding the appropriate Biosafety Level during the processing of TB specimens. We recommend that work with active cultures of TB be done at Biosafety Level 3. When processing clinical specimens for culture or molecular testing such as NAAT, we recommend a minimum of Biosafety Level 2 with Biosafety Level 3 containment procedures [working in a biosafety cabinet, wearing a respirator (i.e. N95), solid-front gowns, and gloves]. The samples used in this program contain levels of mycobacteria that are similar to clinical specimens so the same biosafety precautions should be used.

Overall, composite results for this shipment indicate that laboratories performed well.

We acknowledge the help of the WSLH staff, Dr. David Warshauer, Sue Legois and others in writing this report.

## References

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2. CDC. Nucleic acid amplification tests for tuberculosis. MMWR 1996; 45:950-951.

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9. Band, JD, JI Ward, DW Fraser, et al. Peritonitis due to a *Mycobacterium chelonei*-like organism associated with intermittent chronic peritoneal dialysis. J. Infect. Dis. 145:9-17.

**Figure 1. Primary Classification of Participating Laboratories**

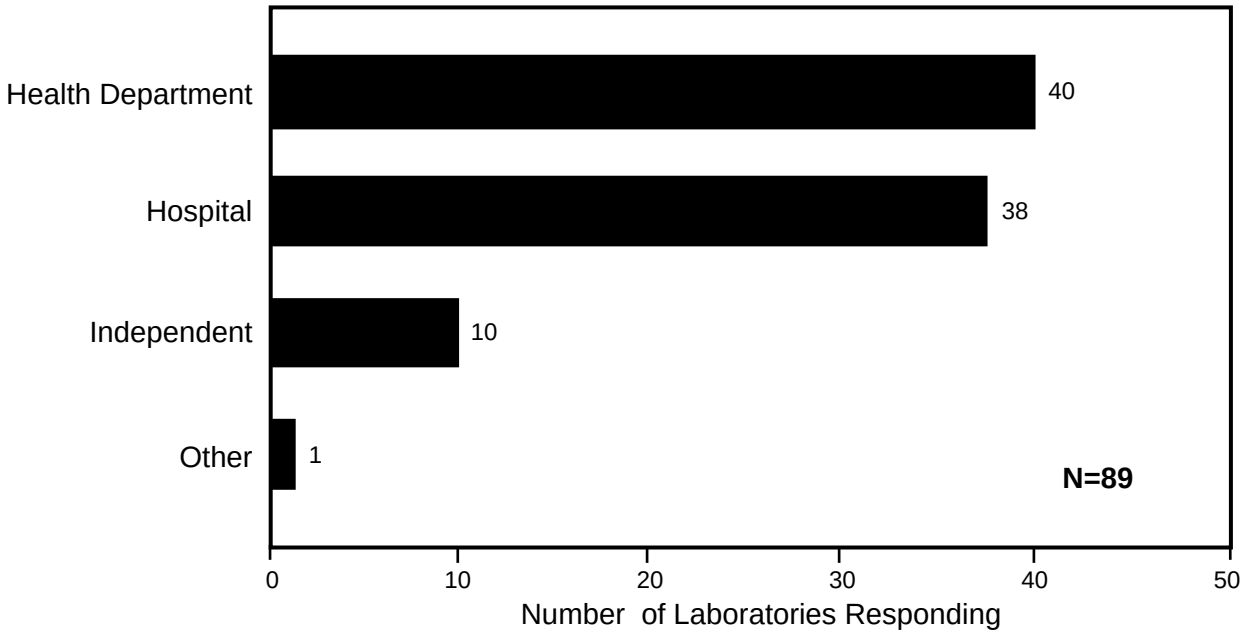
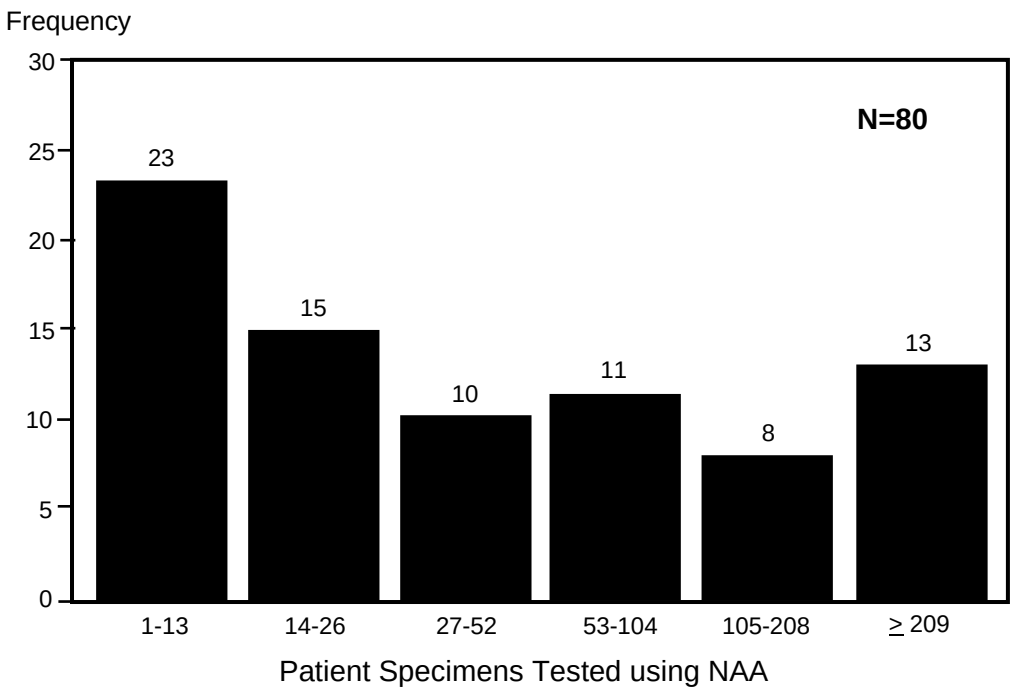



**Figure 2. Number of Patient Specimens Tested for *M.tb* Using TB NAA during the Previous Quarter.***




*See explanation in the analysis.

**Figure 3. Amplification Procedure Used for Direct Detection of *M.tb***

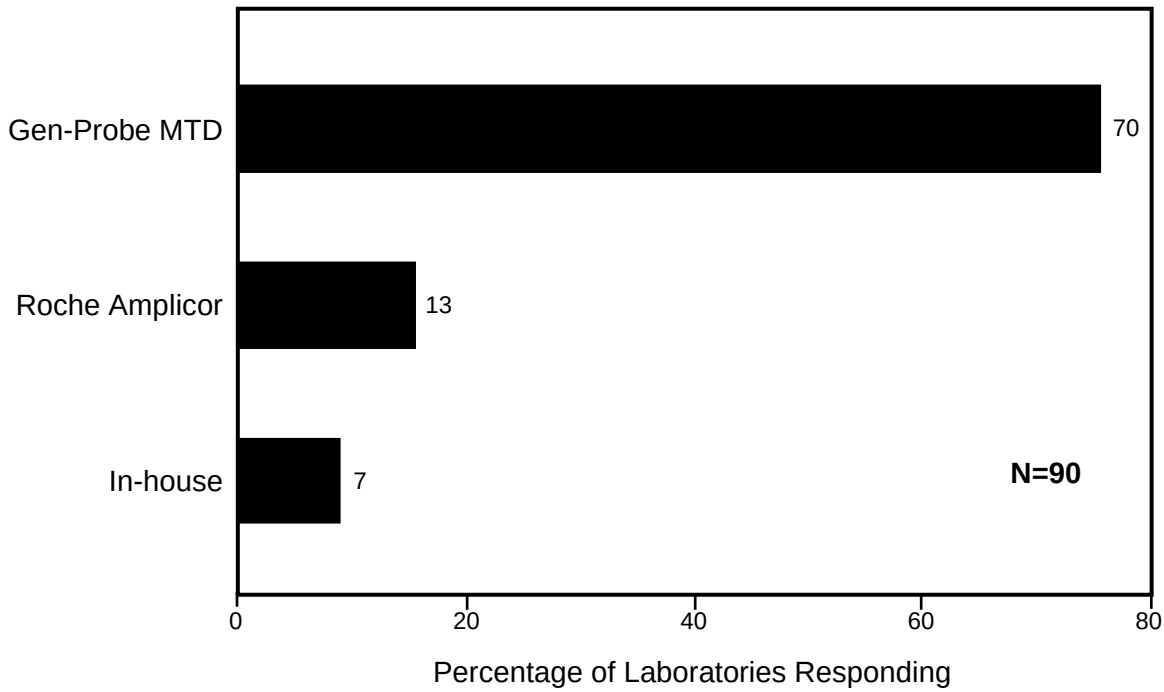



**Figure 4. Biosafety Levels of Participant Laboratories**

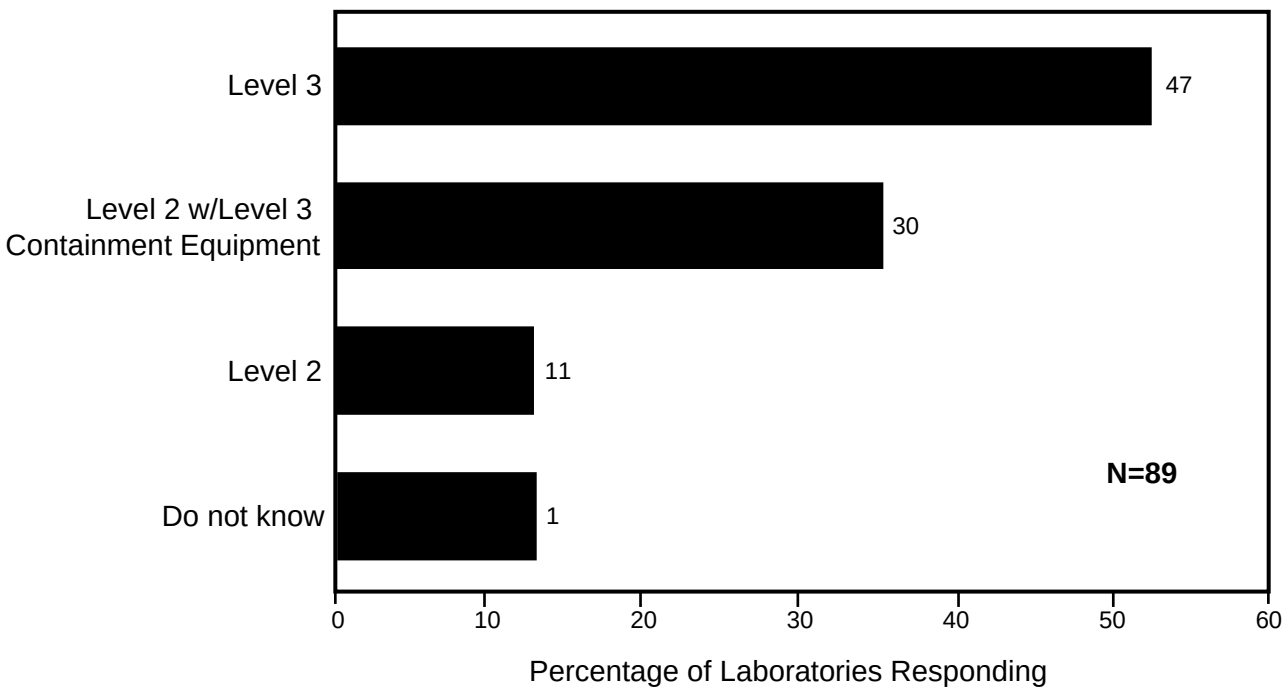


**Figure 5. Is the Biological Safety Cabinet that is Used for TB NAA Testing Used for Other Purposes?**

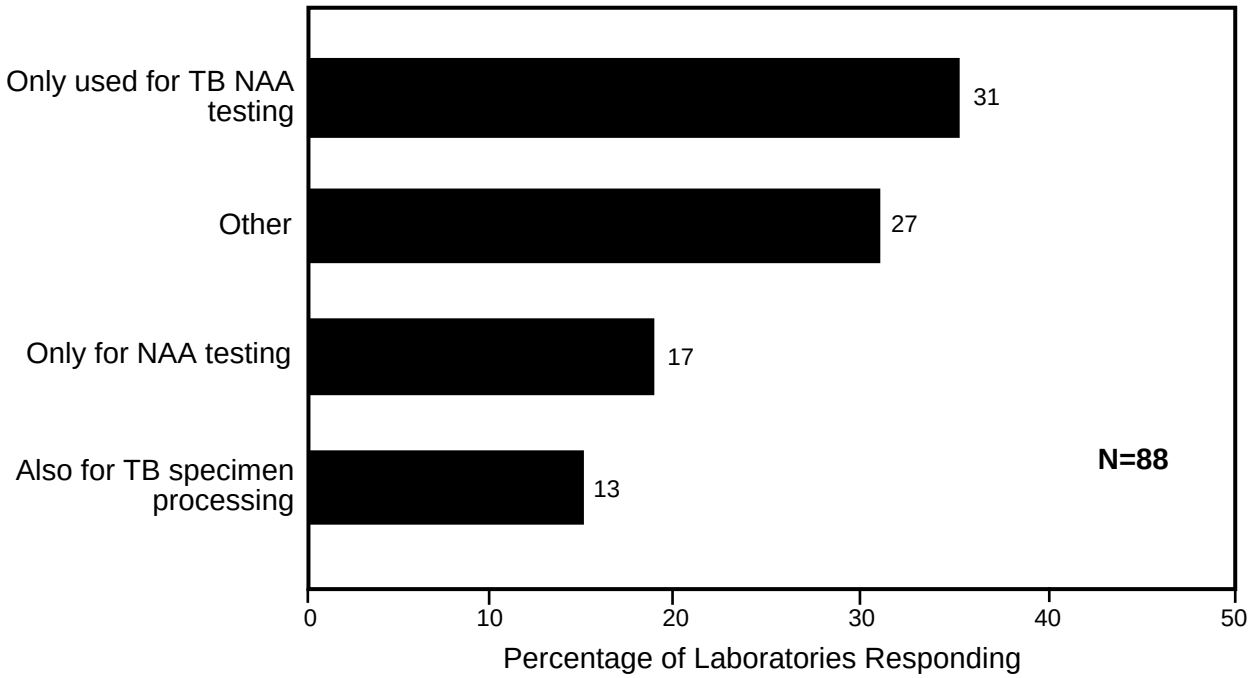



**Figure 6. Use of Uni-directional Workflow by Participating Laboratories**

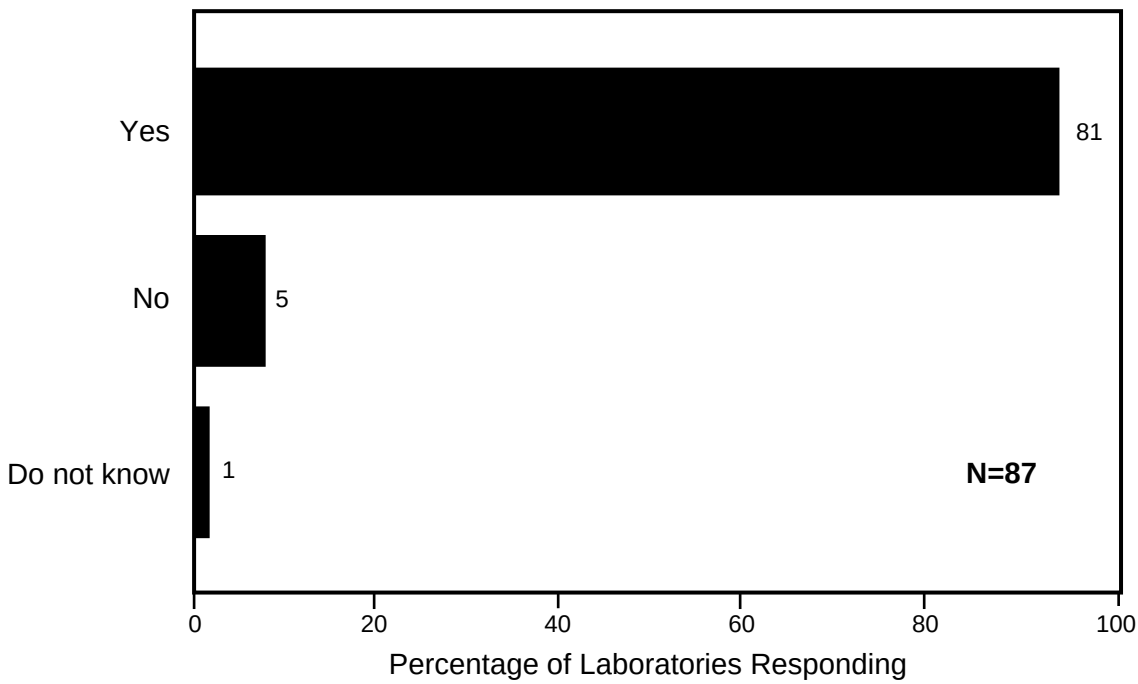
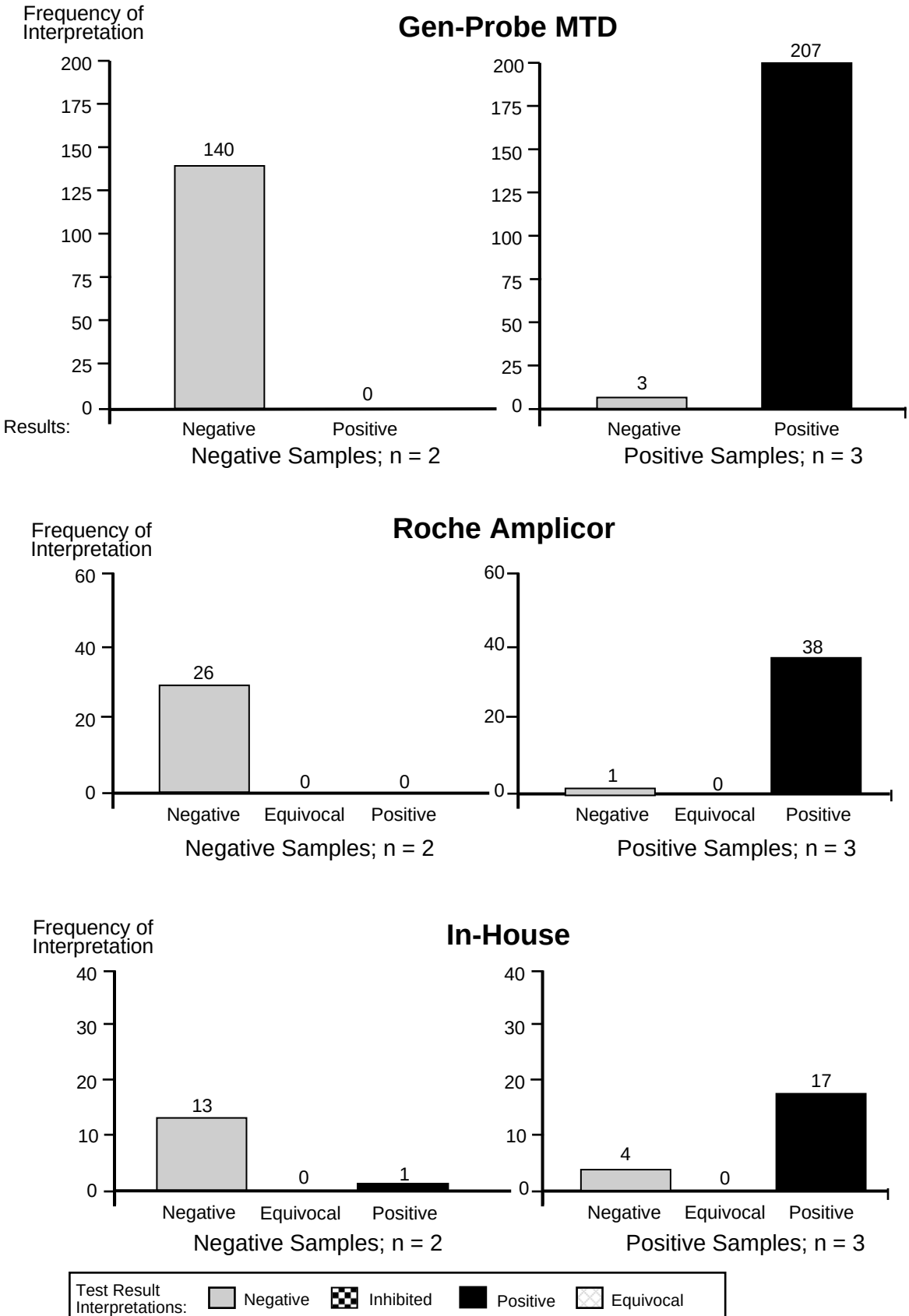
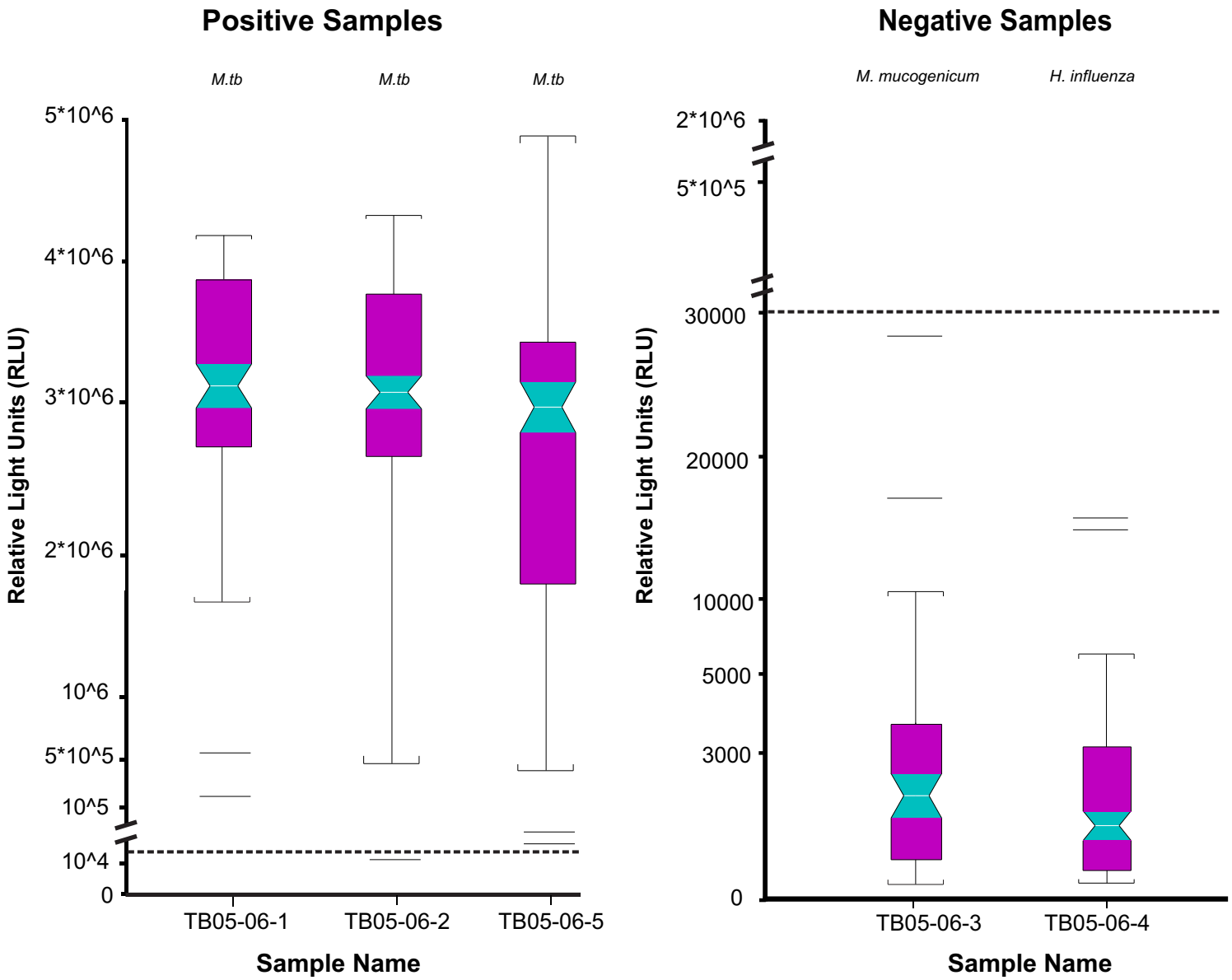


**Figure 7. Frequency of TB NAA Qualitative Test Results by Sample Type for the Gen-Probe MTD, Roche Amplicor, and In-House Methods**

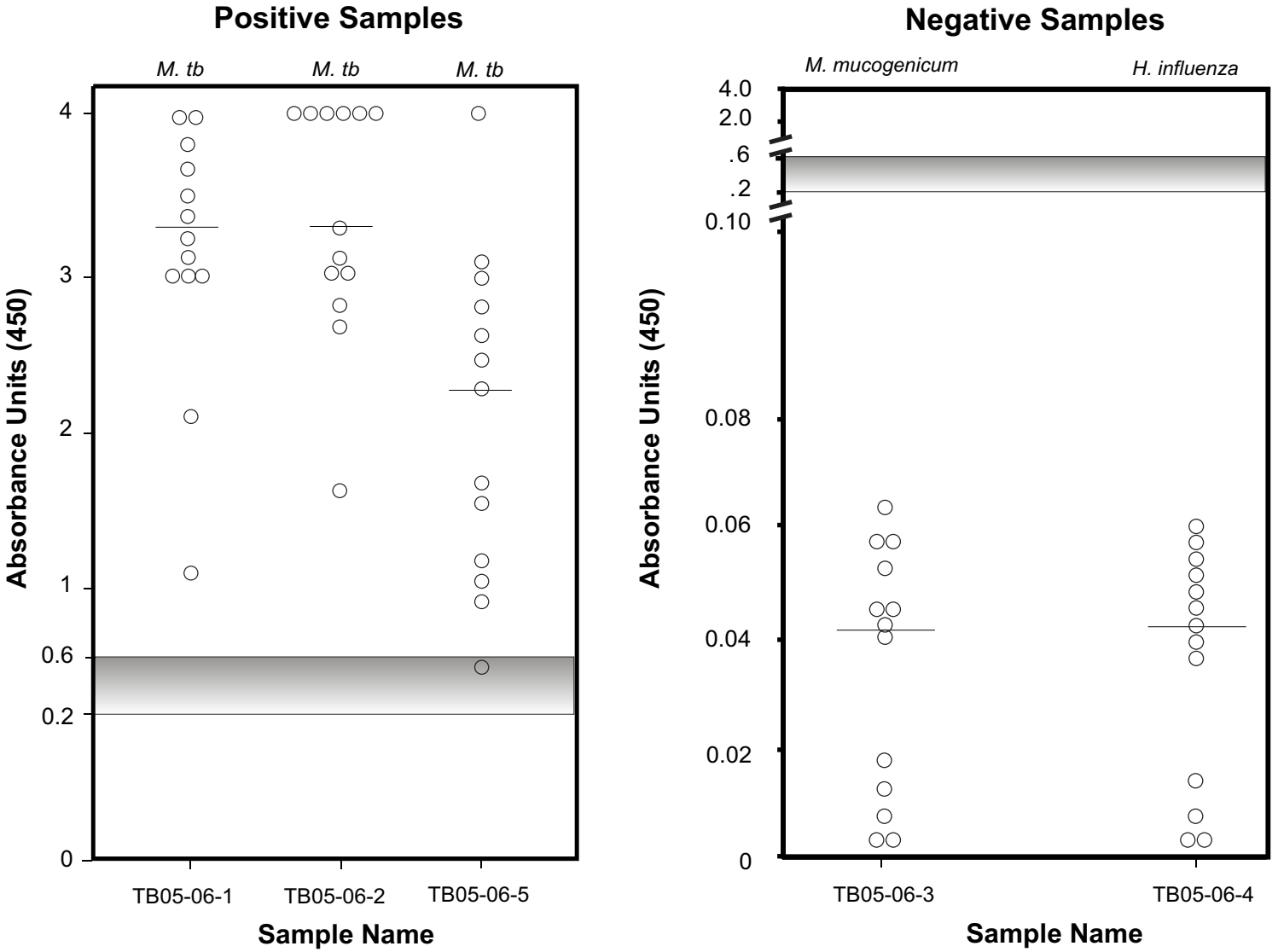


# Figure 8. Quantitative Results for GenProbe® MTD




Note: Dashed line (**---**) represents cut-off between positive and negative values (30,000 RLUs).

# Figure 9. Quantitative Results for Roche Amplicor®




Note: Shaded areas represent equivocal range.

The following tables summarize qualitative results reported by participant laboratories for the June 2005 shipment of samples for the *M. tb* NAA testing performance evaluation program.

Table 1. Sample TB05-06-1 contained *Mycobacterium tuberculosis* ($3.1 \times 10^5$ theoretical cells/ml)

| Test Methods | No. Tests Performed | Positive No. | Positive % | Inhibition Not applicable | Equivocal No. | Equivocal % | Negative No. | Negative % |
|---|---|---|---|---|---|---|---|---|
| Gen-Probe | 70 | 70 | 100.0 | | n/a | n/a | 0 | 0.0 |
| In-house | 7 | 6 | 85.7 | | 0 | 0.0 | 1 | 14.3 |
| Roche | 13 | 13 | 100.0 | | 0 | 0.0 | 0 | 0.0 |
| All methods | 90 | 89 | 98.9 | | 0 | 0.0 | 1 | 1.1 |

Table 2. Sample TB05-06-2 contained *Mycobacterium tuberculosis* ($2.77 \times 10^5$ theoretical cells/ml)

| Test Methods | No. Tests Performed | Positive No. | Positive % | Inhibition No. | Inhibition % | Equivocal No. | Equivocal % | Negative No. | Negative % |
|---|---|---|---|---|---|---|---|---|---|
| Gen-Probe | 70 | 69 | 98.6 | | | n/a | n/a | 1 | 1.4 |
| In-house | 7 | 7 | 100.0 | | | 0 | 0.0 | 0 | 0.0 |
| Roche | 13 | 13 | 100.0 | | | 0 | 0.0 | 0 | 0.0 |
| All methods | 90 | 89 | 98.9 | | | 0 | 0.0 | 1 | 1.1 |

Table 3. Sample TB05-06-3 contained *Mycobacterium mucogenicum* ($3 \times 10^3$ theoretical cells/ml)

| Test Methods | No. Tests Performed | Positive No. | Positive % | Inhibition No. | Inhibition % | Equivocal No. | Equivocal % | Negative No. | Negative % |
|---|---|---|---|---|---|---|---|---|---|
| Gen-Probe | 70 | 0 | 0.0 | | | n/a | n/a | 70 | 100.0 |
| In-house | 7 | 0 | 0.0 | | | 0 | 0.0 | 7 | 100.0 |
| Roche | 13 | 0 | 0.0 | | | 0 | 0.0 | 13 | 100.0 |
| All methods | 90 | 0 | 0.0 | | | 0 | 0.0 | 90 | 100.0 |

Table 4. Sample TB05-06-4 contained *Haemophilus influenzae* ($3 \times 10^4$ theoretical cells/ml)

| Test Methods | No. Tests Performed | Positive No. | Positive % | Inhibition No. | Inhibition % | Equivocal No. | Equivocal % | Negative No. | Negative % |
|---|---|---|---|---|---|---|---|---|---|
| Gen-Probe | 70 | 0 | 0.0 | | | n/a | n/a | 70 | 100.0 |
| In-house | 7 | 1 | 14.3 | | | 0 | 0.0 | 6 | 85.7 |
| Roche | 13 | 0 | 0.0 | | | 0 | 0.0 | 13 | 100.0 |
| All methods | 90 | 1 | 1.1 | | | 0 | 0.0 | 89 | 98.9 |

Table 5. Sample TB05-06-5 contained *Mycobacterium tuberculosis* ($3 \times 10^3$ theoretical cells/ml)

| Test Methods | No. Tests Performed | Positive No. | Positive % | Inhibition Not applicable | Equivocal No. | Equivocal % | Negative No. | Negative % |
|---|---|---|---|---|---|---|---|---|
| Gen-Probe | 70 | 68 | 97.1 | | n/a | n/a | 2 | 2.9 |
| In-house | 7 | 4 | 57.1 | | 0 | 0.0 | 3 | 42.9 |
| Roche | 13 | 12 | 92.3 | | 0 | 0.0 | 1 | 7.7 |
| All methods | 90 | 84 | 93.3 | | 0 | 0.0 | 6 | 6.7 |